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CONTENTS

PAGE

SAUNDERS, D. S. The ovulation cycle in *Glossina morsitans* Westwood (DIPTERA : MUSCIDAE) and a possible method of age determination for female Tsetse Flies by the examination of their ovaries 221–238, 5 figs.

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THE OVULATION CYCLE IN *GLOSSINA MORSITANS* WESTWOOD (DIPTERA : MUSCIDAE) AND A POSSIBLE METHOD OF AGE DETERMINATION FOR FEMALE TSETSE FLIES BY THE EXAMINATION OF THEIR OVARIES

By D. S. Saunders

(*Department of Zoology, University of Edinburgh*)

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With 5 Text-figures

Contents

		page
I.	Introduction	221
II.	Maintenance of the flies and methods of dissection	221
III.	The anatomy of the ovary of *Glossina morsitans* Westwood	223
IV.	The normal ovulation cycle in the fertilised female	224
V.	Ovulation in virgin females	230
VI.	Abnormalities in reproduction associated with laboratory culture	233
VII.	Discussion	234
VIII.	Summary	236
IX.	Acknowledgments	236
X.	References	237

I. Introduction

In a study of the ovulation cycle of *Glossina palpalis* (Robineau-Desvoidy), Helen Mellanby (1937) established that the first egg is ovulated from the right ovary and subsequent eggs alternately from left and right ovaries throughout the life of the fly. A similar cycle was found to occur in *G. morsitans* Westwood and *G. swynnertoni* Austen (Mellanby, K., 1937) and probably also occurs in other species of tsetse flies (Buxton, 1955). These studies were based on the appearance of the ovaries as a whole and on the assumption that each ovary contained a single ovariole. It has been shown by dissection, however, that two ovarioles are present in each ovary of *G. morsitans* (Saunders, 1960) and also of *G. palpalis*, *G. pallidipes* Austen and *G. brevipalpis* Newstead. In this respect the ovary of tsetse flies is similar to that of the Pupipara such as *Melophagus ovinus* L. (Pratt, 1899) and *Hippobosca longipennis* F. (*capensis*) (Detinova, 1955). In view of this discovery the ovulation cycle of *Glossina* calls for a redescription. The present paper is a study of the changes occurring in the ovarioles of *G. morsitans* during the ovulation cycle and also considers the possibility of determining the physiological age of female tsetse flies from the appearance of the ovarioles after ovulation, a method based upon recent Soviet work on *Anopheles maculipennis* Meigen and other blood-sucking Diptera (Detinova, 1959).

II. Maintenance of Flies and Methods of Dissection

The *Glossina morsitans* used in this investigation emerged from pupae collected near Singida, Tanganyika, and flown to Edinburgh.

Newly emerged flies were enclosed singly in glass specimen tubes and kept at a temperature of 26–27° C. and a relative humidity of between 40 and 60 per cent. The tubes were fitted with nylon net tops with a mesh sufficiently open to allow the flies to feed through it. A small circle of blotting paper was stuck to the floor of each

tube to absorb the faeces. The flies were blood-fed on the human hand or the shaved sides of a guinea-pig. Males and virgin females were found to take smaller and more frequent blood-meals than pregnant females. For this reason they were offered a blood-meal on alternate days, or, if they refused, every subsequent day until they fed. Pregnant females were found to take much larger blood-meals at longer intervals, most of them feeding to repletion every third or fourth day. Frequency of feeding, therefore, was adjusted to the apparent physiological need of the flies.

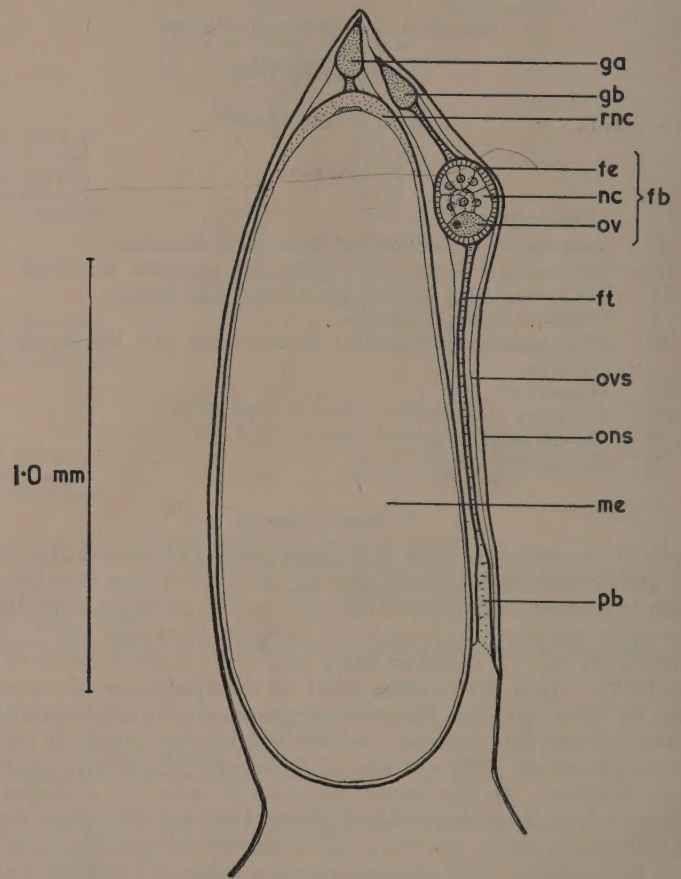

Fig. 1.—Right ovary of 7-day-old *Glossina morsitans*. (*fb*, follicle in ovariole *B* ; *fe*, follicular epithelium ; *ft*, follicular tube ; *ga*, germarium of ovariole *A* ; *gb*, germarium of ovariole *B* ; *me*, mature egg in ovariole *A* ; *nc*, nurse cells ; *ons*, ovarian sheath ; *ov*, ovum ; *ovs*, ovariole sheath ; *pb*, pedicel of ovariole *B* ; *rnc*, remains of nurse cells.)

Females were mated when about three to five days old with older and, if possible, virgin males in the tubes in which they were kept. Some females were mated by releasing them into a cage of older males, but this was discontinued because poor feeding of the males in the cage caused a loss in their potency. Mellanby (1936) considered that insemination was more likely to have taken place with couples that remain *in copula* for more than half an hour. In order to ensure a large proportion of impregnated flies, therefore, any females which refused to copulate or which sep-

arated soon after copulation were placed with another male. Whether insemination had taken place could be checked during dissection by an examination of the spermathecae, which clearly show any contained spermatozoa (Mellanby, 1936).

Dissections were carried out in 0·9 per cent. saline on a microscope slide. The reproductive organs of the female flies were removed in the saline and stripped of fat bodies and the spermathecae examined for spermatozoa; the uterus was also examined to determine whether it contained an egg or a first, second or third instar larva. Both ovaries were then stripped of the ovarian and ovariole sheaths with a fine needle, thus the follicular tubes of the ovarioles were freed. The lengths of the egg follicles were measured by means of an ocular micrometer and the ovarioles mounted in a drop of polyvinyl-lactophenol containing a little lignin pink. This mountant produced a permanent preparation without distorting the delicate follicular tubes and follicles.

III. The Anatomy of the Ovary of Glossina morsitans Westwood

The general morphology of the female reproductive system was described by Stuhlman (1907) and Roubaud (1909) and does not need redescription. These authors, however, considered that each ovary consisted of a single ovariole, a view shared by Hagen (1951), who studied the anatomy of the ovaries of *G. tachinoides* Westwood. As the ovaries of four species of *Glossina* (*G. morsitans*, *G. palpalis*, *G. pallidipes* and *G. brevipalpis*) have been shown to contain two ovarioles, a more detailed description of the ovary is thought necessary.

The anatomy of the ovaries of *G. morsitans* was established by dissection and by the examination of serial sections stained in Heidenhain's iron haematoxylin. Each ovary (fig. 1) is invested in an ovarian sheath (*ons*) which is well supplied with muscle strands and tracheae, and contains two polytrophic ovarioles. Each ovariole is enclosed in an extremely fine ovariole sheath (*ovs*) which is often removed with the outer ovarian sheath in dissection; within the ovariole sheath is the follicular tube which encloses the germarium and the egg follicle. The posterior end of the follicular tube (the pedicel) is somewhat expanded and is fused to the internal lining of the ovary. The morphology of the contents of the follicular tube is best described by a brief account of the developmental processes taking place within it. Cells in the germarium give rise, by oogenesis, to eight daughter cells, one of which becomes the oocyte and the other seven the nurse or nutritive cells (fig. 2, *C*). This group of cells constitutes a young egg follicle which becomes covered with a layer of follicular epithelium and descends from the germarium into the lumen of the follicular tube. Older follicles contain 14 nurse cells (fig. 2, *A*). Verhein (1921) showed that the follicles of *Musca domestica* L. contain an ovum and 15 nurse cells, the result of four mitoses. The 14 nurse cells in *G. morsitans* are considered to arise by a subsequent division of the seven original nurse cells in the newly descended follicle. In the follicular tube the oocyte develops by a deposition of yolk around its nucleus at the expense of the nurse cells and eventually becomes a mature ovum covered by a chorion produced by the follicular epithelium. When the egg is fully developed it projects from the posterior end of the follicular tube; it is later ovulated, the remnants of the nurse cells and the follicular epithelium (the follicular relic) being left behind in the follicular tube. In the normal ovariole of *G. morsitans* there is never more than one egg follicle in the ovariole at any one time, the second follicle descending from the germarium several days after the ovulation of the first egg.

The four ovarioles of a newly emerged *G. morsitans* (fig. 2) are all at different stages of development. The largest egg follicle is in one of the ovarioles (*A*) in the right ovary; this follicle is about 0·48 mm. in length, a third of which is ovum and yolk. The next most advanced follicle is in one of the ovarioles (*C*) in the left ovary. This follicle is about 0·22 mm. in length and shows only the beginnings of yolk

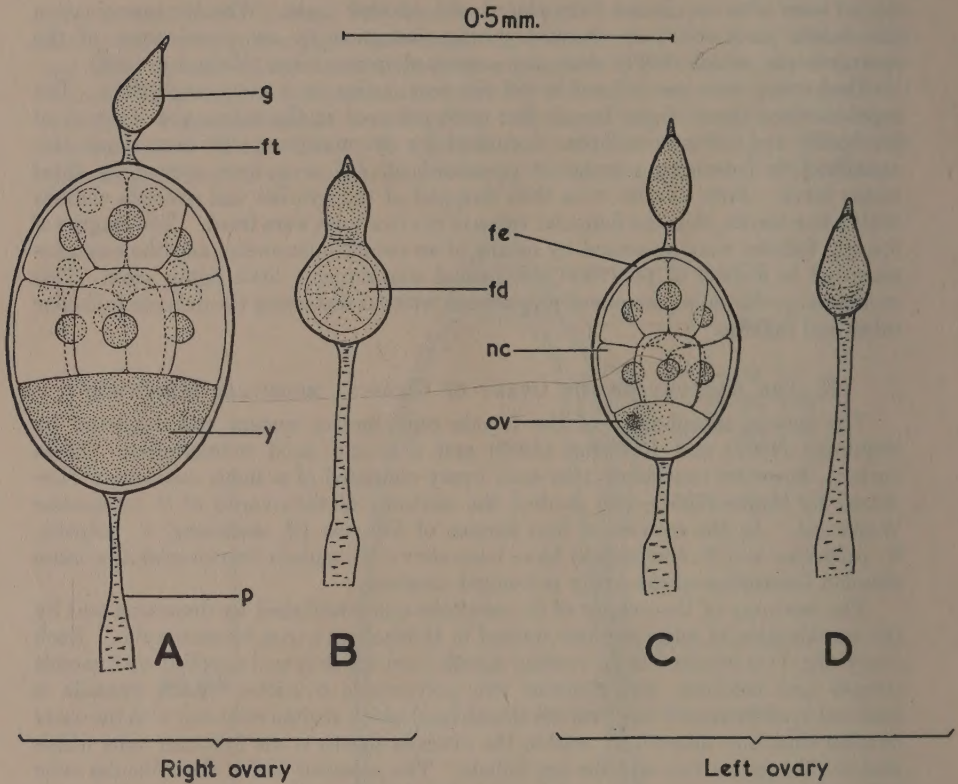

FIG. 2.—The four ovarioles of a newly-emerged *Glossina morsitans* removed from the ovaries, (ovariole sheaths removed in dissection). (*fd*, follicle descending from germarium; *fe*, follicular epithelium; *ft*, follicular tube; *g*, germarium; *nc*, nurse cells; *ov*, ovum; *p*, pedicel; *y*, yolk.) (Terminology after Bertram (1959).)

deposition. The second ovariole in the right ovary (*B*) shows the follicle just descending from the germarium, and the second ovariole in the left ovary (*D*) shows only the germarium and no signs of follicle development. The further development of the follicles is described below.

IV. The Normal Ovulation Cycle in the Fertilised Female

At 26–27° C. the first egg is ovulated from the right ovary of a normal fertilised female *G. morsitans* on the eighth or ninth day, and the first larva, which develops from this egg, is deposited on about the 19th or 20th day. Subsequent larvae are then produced at about ten-day intervals from eggs ovulated alternately from left and right ovaries. Each blood-meal taken by the fly contributes to a continuous development of the egg follicles. This is unlike the situation in the Nematocera and Brachycera in which one blood-meal normally leads to one oviposition, a phenomenon known as gonotrophic concordance. The course of development of the egg follicles in the four ovarioles is described below and illustrated in figures 3 and 4.

The first egg follicle to reach maturity and the one which initially shows the greatest rate of development is that in ovariole *A*, which was the largest follicle at emergence. The length of this follicle increases from about 0·48 mm. at emergence

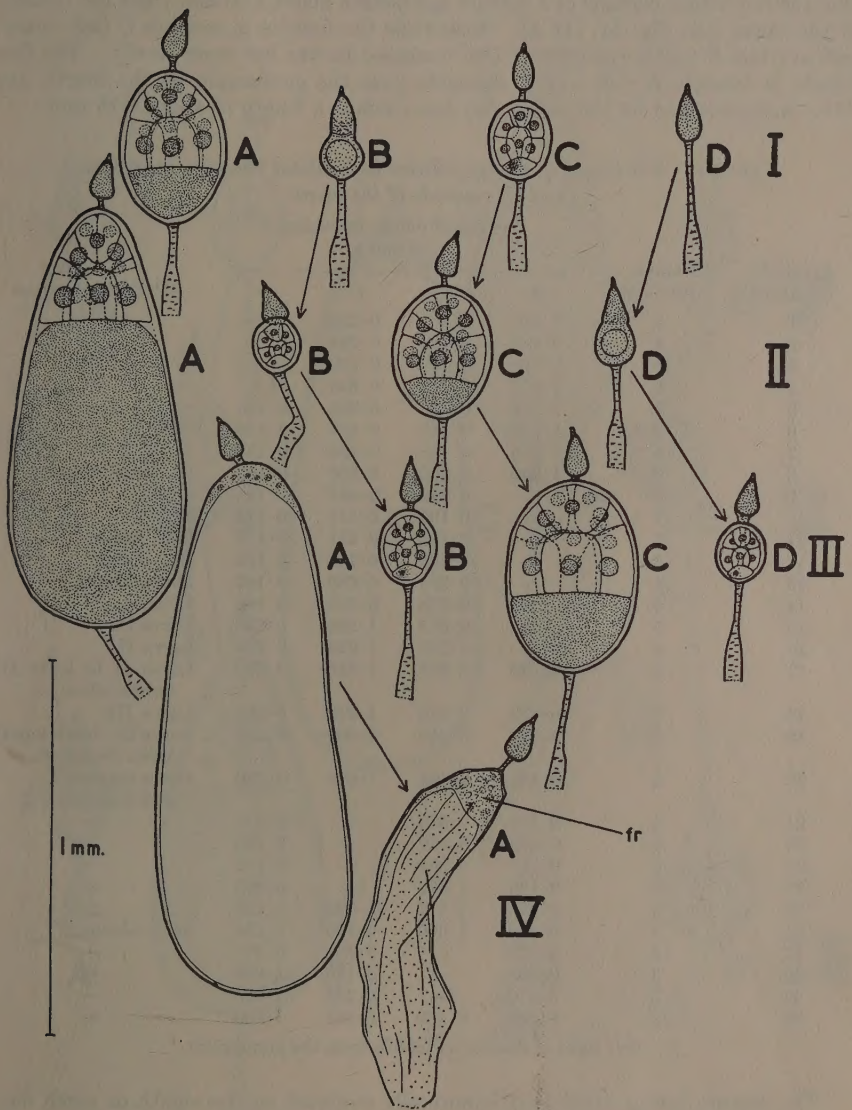

FIG. 3*a*.—The ovulation cycle in *Glossina morsitans*.

(I) The four ovarioles in a newly emerged female.

(II) The four ovarioles in a 4-day-old female : first signs of follicle in ovariole *D*.

(III) The four ovarioles in a 7-day-old female : ovariole *A* containing mature egg ; follicle *D* descended from germarium.

(IV) Ovariole *A* immediately after ovulation. (*fr*, follicular relic.)

to the full length of about 1·60 mm. on the seventh or eighth day (Table I). At this age the follicle consists of a mature egg (length about 1·50 mm.) and the remains of the nurse cells (fig. 3*a*, III *A*). Meanwhile the follicles in ovariole *C* (left ovary) and ovariole *B* (right ovary) have also increased in size but more slowly. The first follicle in ovariole *D* (left ovary) descends from the germarium on the fourth day after emergence and by the eighth day has reached a length of about 0·16 mm.

TABLE I.—*The length of the egg follicles in fertilised* Glossina morsitans *and the contents of the uterus*

Age of fly (in days)	Number dissected	Length of follicle in ovariole (in mm.) *A*	*B*	*C*	*D*	Contents of uterus
0–1	5	0·486	f.s.	0·226	—	—
2	4	0·855	0·126	0·292	—	—
3	4	0·940	0·130	0·280	—	—
4	5	1·052	0·174	0·336	f.s.	—
5	4	1·252	0·172	0·365	0·130	—
6	4	1·350	0·160	0·405	0·140	—
7	4	1·355	0·185	0·465	0·155	—
8	6	1·600	0·200	0·506	0·165	1*st ovulation*—egg.
9	6	—	0·180	0·491	0·166	Egg.
10	7	—	0·193	0·541	0·174	Egg.
11	7	—	0·194	0·611	0·178	Egg—larva.
12	5	—	0·222	0·736	0·176	Egg—larva.
13	4	—	0·224	0·680	0·182	Larva I or II.
14	5	f.s.	0·238	0·802	0·192	Ditto.
15	5	f.s.	0·313	1·298	0·228	Larva II.
16	4	f.s.	0·272	1·225	0·220	Larva II.
17	5	0·130	0·374	1·516	0·230	Larva II to larva III (white lobes).
18	3	0·133	0·416	1·570	0·263	Larva III.
19	5	0·154	0·410	1·600	0·230	Larva III (black lobes): larva deposited.
20	3	0·153	0·463	1·600	0·250	Larva deposited *2nd ovulation.*
21	2	0·185	0·535	—	0·270	—
23	4	0·169	0·557	—	0·230	—
25	4	0·217	0·960	—	0·272	—
27	3	0·190	1·270	f.s.	0·293	—
29	3	0·219	1·516	0·090	0·436	—
31	4	0·280	1·600	0·125	0·552	3*rd ovulation.*
33	2	0·275	—	0·180	0·870	—
35	3	0·293	—	0·190	1·093	—
37	5	0·370	f.s.	0·222	1·306	—
39	3	0·433	0·130	0·243	1·600	—

f.s., first signs of descent of follicle from the germarium.

The mature egg in ovariole *A* is normally ovulated on the eighth or ninth day, although some females retain this egg until about the twelfth day after emergence, which causes a corresponding delay in the deposition of the first larva. Immediately after ovulation, the follicular tube of this ovariole, which was greatly stretched by the growth and ovulation of the egg, is an open expanded sac (fig. 3*a*, IV). This is equivalent to the corresponding stage in the ovulation cycle of *Anopheles maculipennis*, described by Detinova (1959). The remnants of the nurse cells and the follicular epithelium (the follicular relic) remain behind in the follicular tube after ovulation and can be seen as a pale yellow pigmented area at the anterior end of the dilatation. Within the next few days the follicular tube contracts and seems to become more delicate. The follicular relic is passed out of the ovariole and remains for a while at

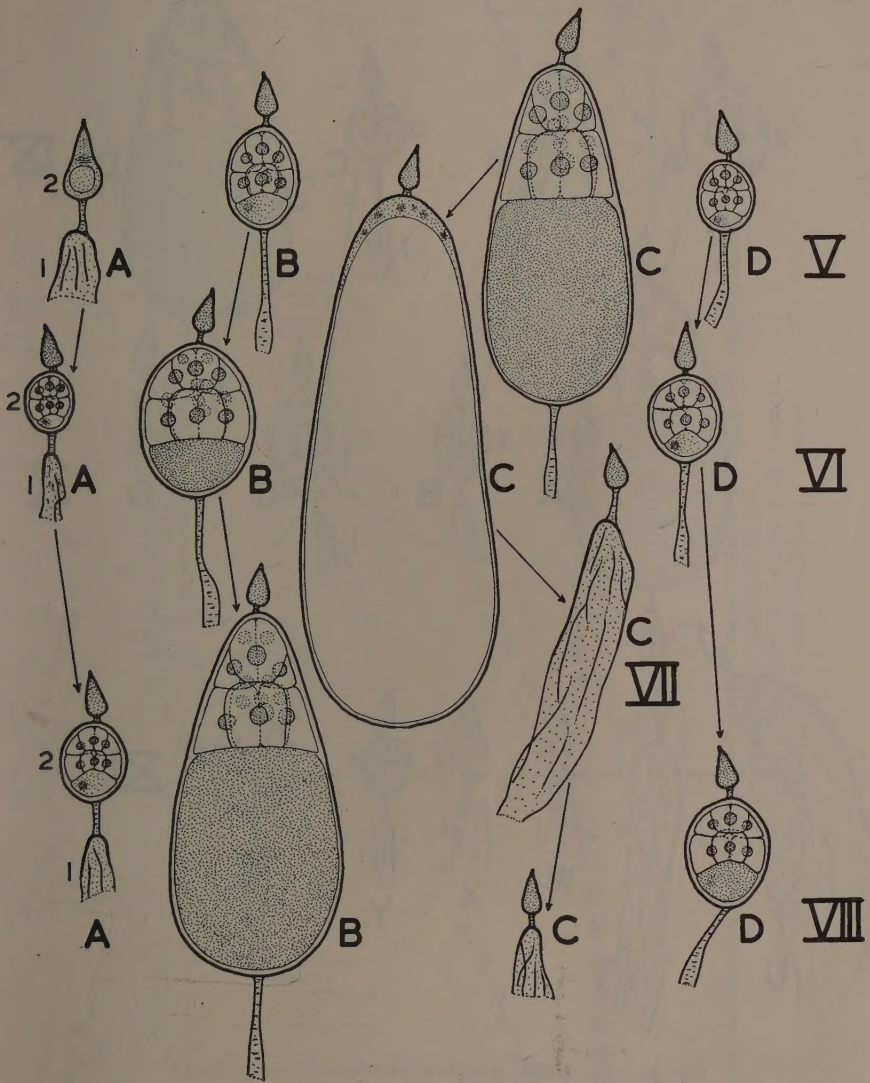

FIG. 3*b*.—The ovulation cycle in *Glossina morsitans* (contd.).

(V) The four ovarioles of a 14-day-old female : largest egg follicle in ovariole *C* ; ovariole *A* showing " funicle stump " and first signs of second follicle.

(VI) The four ovarioles of a 20-day-old female : ovariole *C* containing mature egg ; ovariole *A*, a fully descended follicle.

(VII) Ovariole *C* 24 hours after ovulation, follicular relic passed out.

(VIII) The four ovarioles of a 25-day-old female : largest egg follicle in ovariole *B* ; " funicle stumps " on ovarioles *A* and C.

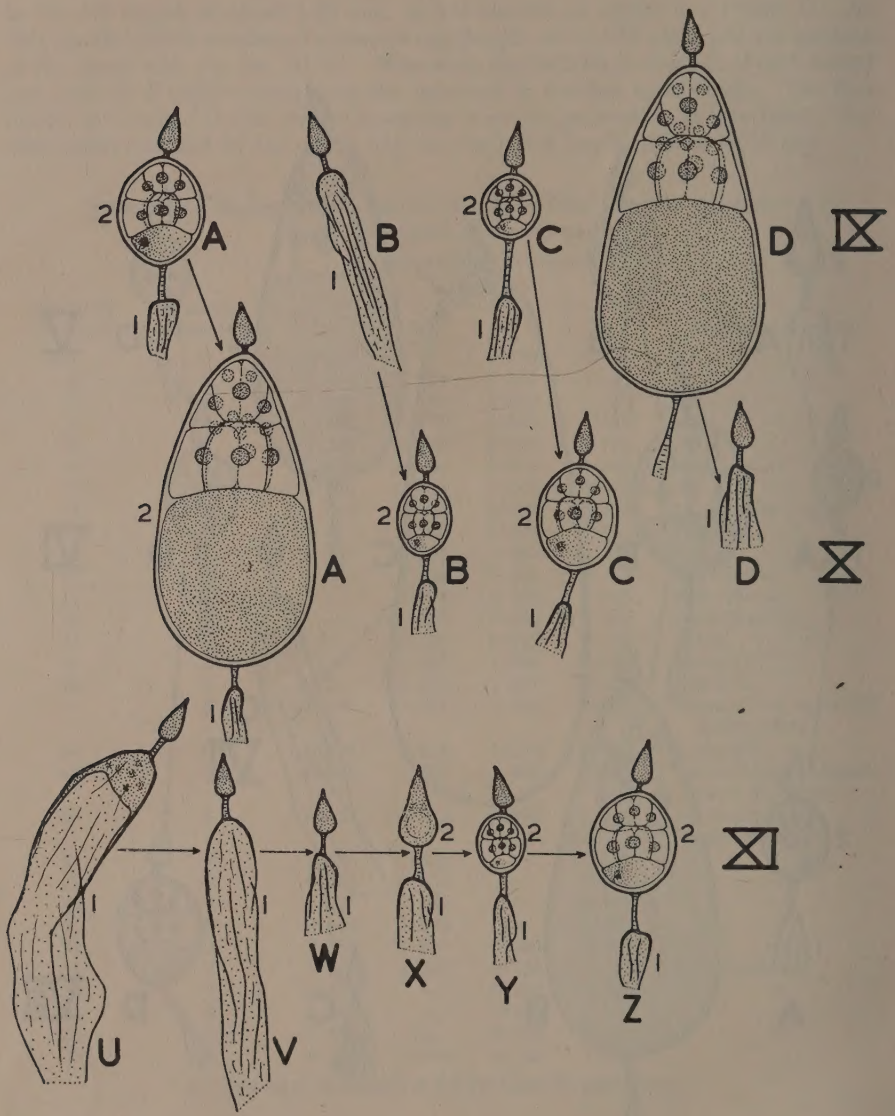

FIG. 3*c*.—The ovulation cycle in *Glossina morsitans* (contd.).

(IX) The four ovarioles of a 33-day-old female : largest egg in ovariole *D* ; " funicle stumps " on ovarioles *A*, *B* and *C*.

(X) The four ovarioles of a 43-day-old female : largest egg in ovariole *A* (second follicle) ; " funicle stumps " on all four ovarioles.

(XI) Changes occurring in an ovariole after ovulation : *U*, immediately after ovulation, follicular tube containing follicular relic ; *V*, 24 hours after ovulation, follicular tube contracting, follicular relic passed out ; *W*, 3 days after ovulation, most of expanded part of follicular tube detached ; *X*, 6 days after ovulation, first signs of second follicle in germarium ; *Y*, 12 days after ovulation, second follicle descended from germarium ; *Z*, 25 days after ovulation.

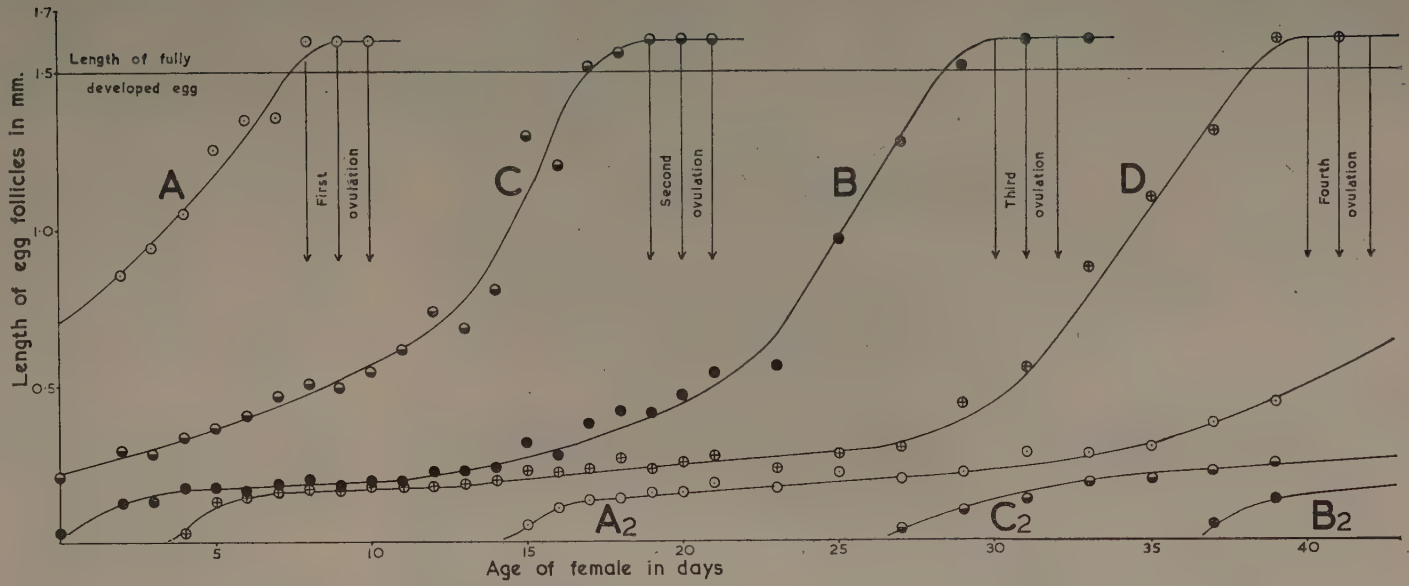

FIG. 4.—The lengths of the egg follicles in *Glossina morsitans*, showing the sequence of egg development and ovulation at 26–27° C.
(*A*), (*B*), (*C*), (*D*) : first follicles in ovarioles *A*, *B*, *C* and *D*.
(*A*2), (*B*2), (*C*2) : second follicles in ovarioles *A*, *B* and *C*.

the posterior end of the ovary close to the junction with the uterus. Occasionally, in older females which have completed several ovulation cycles, quite conspicuous groups of these follicular relics may be seen in this position, corresponding to those described in *Stomoxys calcitrans* L. by Kuzina (1950).

In ovariole *A* the first signs of the descent of the second follicle from the germarium are shown 14 or 15 days after the emergence of the fly (fig. 3*b*, V *A*). At this stage the dilation left after ovulation of the first egg ceases to resemble that in *Anopheles* and appears as a small, extremely delicate structure on the posterior end of the follicular tube. This structure appears to be the broken anterior end of the large sac seen immediately after the passage of the first egg. At an early stage in the investigation it was thought that this was the result of clumsy dissection, but it has now been studied more carefully and by serial sections. It appears that the pedicel becomes completely detached from the internal lining of the ovary during ovulation and subsequently a greater part of the expanded follicular tube is also detached, possibly because of the great size of the mature egg in relation to the ovary and because the germarium of ovariole *A* is pushed towards the anterior end of the ovary by the growth of the follicle in ovariole *B*. The result is that the follicular tube is attached to the inside of the ovary at its anterior end above the germarium, but the posterior end lies freely within the ovariole sheath. The small piece of tissue remaining on the free end of the follicular tube, which closely resembles the structures described as " funicle stumps " in parous *Anopheles gambiae* Giles (Lewis, 1958), is evidence of ovulation.

The cycle described for ovariole *A* is repeated in the other three ovarioles in such a way that a sequence of ovulation occurs. At 26–27° C. the follicle in ovariole *C* reaches maturity and is ovulated on about the 20th day after emergence (fig. 3*b*, VI *C*), and the follicles in *B* and *D* on about the 30th and 40th days. By the 50th day, or thereabouts, the second follicle in ovariole *A* is mature and is then ovulated.

It can be seen from figure 4 that the four follicles do not develop at the same rate at the same time. Up to about the tenth day follicle *A* is developing most rapidly, follicle *C* to a lesser extent and follicles *B* and *D* hardly at all. After ovulation of the first egg from ovariole *A*, the rate of development of the follicle in ovariole *C* increases so that it takes the place of follicle *A* in the developmental sequence. The rate of development of follicle *B* increases at this time, although by a lesser amount, and follicle *D* and the second follicle in *A* continue their slow development. After the ovulation of egg *C* (about 20 days), the rate of development of follicle *B* is sharply increased. It follows from these observations that ovulation causes an increase in the rate of development of the other follicles, the amount of increase depending upon their order in the sequence of ovulation. This sequence of development is probably under hormone control.

V. Ovulation in Virgin Females

A proportion (16·9 per cent.) of the female *G. morsitans* which copulated during these experiments were not inseminated and a smaller number were kept apart from the males; as there was no difference in the ovarian development of these two categories of flies they are all considered as virgins. Forty-eight virgin flies were dissected and the changes in their ovaries are described below.

Helen Mellanby (1937) showed that there was a delay in ovulation in virgin *G. palpalis* and that old individuals may have several mature eggs in their ovaries. Working with the same species, Vanderplank (1947) found that the first egg may be retained up to the 20th or 22nd day, but once ovulation had commenced it proceeded normally. It would appear from these observations that virgin *G. palpalis* have the tendency to retain mature eggs, at least up to the 22nd day. This does not seem to be the case, however, in virgin *G. morsitans*, and the majority of those dissected in

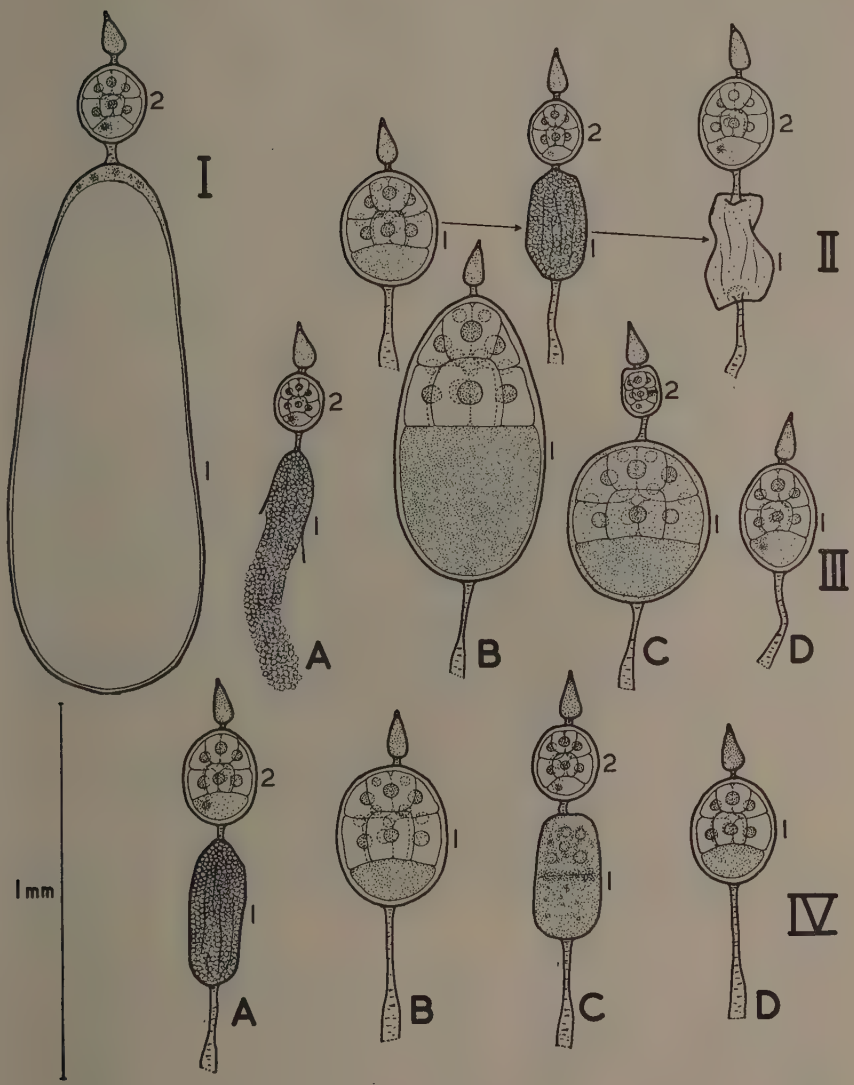

Fig. 5.—(I) Ovariole in virgin *G. morsitans*, showing retention of mature egg and development of second follicle.

(II) Stages in the degeneration of an egg follicle.

(III) Ovarioles of a 15-day-old fertilised *G. morsitans* with the first follicles in ovarioles *A* and *C* degenerated, showing accelerated development of the other follicles. (Degenerated follicle in *A* is broken.)

(IV) Ovarioles of an 18-day-old female showing follicular degeneration.

the present investigation behaved almost normally and had ovulated the first egg by the eighth or ninth day. Only two flies were found to have retained this egg up to the 21st day. In these individuals, the second egg (in ovariole *C*) and the second follicle in ovariole *A* had also started to develop, so that two mature eggs were present (one in the right and one in the left ovary), and ovariole *A* showed the abnormal condition of two egg follicles in the same ovariole at the same time (fig. 5, *I*).

Several virgin females were dissected in which two eggs had been ovulated by the 16th day; in some cases the first egg was not in the uterus and had presumably been extruded. Very few of the older virgins dissected (ages between 35 and 53 days) showed any tendency to retain eggs. Most of them ovulated when the eggs reached maturity and a few had ovulated more eggs than fertilised females of the same age. This may be explained by the fact that ovulation is delayed in pregnant females by the presence of the larva in the uterus and virgin females have no such larva to impede ovulation. On the whole, the majority of virgin *G. morsitans* do not retain fully developed eggs and insemination cannot be the factor controlling ovulation.

Virgin female *G. morsitans* differ from fertilised females in the degree of development of the egg follicles. Table II shows the lengths of the follicles in ovarioles *B*, *C* and *D* compared with the same follicles in fertilised flies during the period (9–21 days) when the latter are producing their first larva. It will be seen that the follicles in the virgins are generally smaller than those in fertilised females, a difference most marked in ovariole *C* which is developing at the greatest rate at this time. During pregnancy, fertilised females are taking larger blood-meals than virgins, primarily in order to nurture the developing larva. The difference in size between the follicles in virgin and fertilised flies may possibly be associated with the size of the meal. This difference has been tested for significance by the following method: for each day's data, the mean follicle length for virgin females was subtracted from the length of the egg follicle in each individual virgin and fertilised fly. The adjusted readings were then treated as two samples (one of virgin flies and one of fertilised flies), in an analysis of variance (*see* Table II).

TABLE II.—*The length of the egg follicles of* Glossina morsitans, *showing that they are larger in pregnant females than in virgins of the same age*

Age of females (in days)	Number dissected		Length of egg follicles (mm.)					
			Ovariole B		Ovariole C		Ovariole D	
	P	*V*	*P*	*V*	*P*	*V*	*P*	*V*
9	6	5	0·180	0·190	0·491	0·450	0·166	0·150
10	7	3	0·193	0·230	0·541	0·546	0·174	0·186
11	7	3	0·194	0·190	0·611	0·490	0·178	0·156
12	5	4	0·222	0·230	0·736	0·692	0·176	0·175
13	4	4	0·224	0·227	0·680	0·670	0·182	0·190
14	5	3	0·238	0·206	0·802	0·596	0·192	0·176
15	5	3	0·313	0·273	1·298	1·196	0·228	0·203
16	4	4	0·272	0·307	1·225	1·295	0·220	0·197
17	5	3	0·374	0·340	1·516	1·286	0·230	0·196
18	3	4	0·416	0·360	1·570	1·447	0·263	0·240
19	5	3	0·410	0·320	1·600	1·440	0·230	0·245
20	3	4	0·463	0·480	1·600	1·600	0·250	0·218
21	2	2	0·535	0·485	1·600	1·600	0·270	0·300
			F value = 1·866 $P = <0{\cdot}2$ and $>0{\cdot}1$		*F* value = 5·823 $P = <0{\cdot}05$ and $>0{\cdot}01$		*F* value = 4·340 $P = <0{\cdot}05$ and $>0{\cdot}01$	

P, pregnant females.
V, virgin females.

VI. Abnormalities in Reproduction Associated with Laboratory Culture

It is well known that the abnormal conditions in the laboratory affect the physiology of tsetse flies. Flies kept individually in small glass tubes suffer from lack of " exercise " and can only feed at times convenient to the experimenter. Jackson (1936) showed that captive *G. morsitans* build up less fat than wild flies and much of the blood-meal remains undigested in the gut for a considerable time. Because of the method of feeding in individual tubes, flies may not always be able to feed when they have the greatest physiological need to do so. This can either lead to death by starvation, as many flies which " miss " a blood-meal become too weak to take a subsequent one, or to the taking of frequent incomplete meals which are thought to interfere with normal reproduction (Foster, 1957). *G. morsitans*, although feeding frequently upon man in nature, shows a definite preference for "game ", especially Suidae (Weitz and Jackson, 1955). It is possible, therefore, that the reproductive physiology of flies maintained in the laboratory on man or guinea-pig may suffer because of " unsuitable " blood.

The difficulties associated with mating tsetse flies in the laboratory are equally well known. In the field, Vanderplank (1947, 1948) showed that practically all female *G. swynnertoni* and *G. pallidipes*, and all of a smaller number of *G. austeni* Newstead and *G. morsitans orientalis* Vanderplank were inseminated ; Mellanby (1936) showed a similar high proportion of fertilised females in a wild population of *G. palpalis fuscipes* Newstead. This also occurs when the population is greatly reduced by successful control programmes (Teesdale, 1940 ; Jackson, 1949). In the laboratory, however, even when the flies are mated when the sexes are apparently most attractive to one another, the percentage of inseminated females is much lower ; Mellanby (1936) recorded 75–90 per cent. of laboratory kept *G. palpalis fuscipes* becoming successfully fertilised. In the present investigation, out of a total of 367 *G. morsitans* mated, only 83·1 per cent. were inseminated.

The artificial conditions in the laboratory have several effects upon the reproductive cycle. The most apparent of these is the abortion of immature larvae, most probably caused by insufficient feeding (Mellanby, H., 1937). In the normal fly, ovulation does not occur until after the birth of a larva, even if the egg has been fully developed for several days ; presumably the egg is retained whilst the larva is within the uterus. In flies which abort their larvae the next egg is ovulated at an earlier date. If the next egg is already fully developed ovulation will occur, as in the normal female, within the first 24 hours after the clearance of the uterus. In the case of very early abortions the egg will be ovulated as soon as it matures. In either case, the next larva is produced sooner than that of a normal female under the same conditions.

Although normal female tsetse flies produce larvae at regular intervals (at 26–27° C. *G. morsitans* produces the first larva after 20 days, and then subsequent larvae at ten-day intervals), several authors have reported irregularities in the production of larvae from laboratory-kept flies. Vanderplank (1948) noted that small proportions of fertilised *G. morsitans* and *G. swynnertoni* do not produce larvae even though they survive long enough to do so, and Potts (1933) kept numbers of *G. morsitans* in cages and recorded an average pupal production of only about one every 50 days. Buxton and Lewis (1934) reported similar low production of pupae from *G. tachinoides* Westwood. Much of this low pupal production must be due to the inclusion in the results of unfertilised females and also to abortions caused by insufficient feeding. Buxton and Lewis were not of the opinion that a single female could have an intermittent reproductive cycle, a fact which could also help to explain these low reproductive rates. In the present investigation, however, several *G. morsitans* were found to have an intermittent larval production, caused by degeneration of egg follicles within their ovarioles.

Follicles in all stages of degeneration have been found. Early signs are the cessation of growth and the ovum, nurse cells and follicular epithelium becoming darker and more granular in appearance. Degeneration eventually results in the complete breakdown of the follicle cells, the degeneration products being passed out leaving a well-defined dilatation in the follicular tube (fig. 5, II). These dilatations left after follicular degeneration may easily be distinguished from those left in the follicular tube after ovulation by the presence of the pedicel.

There is some evidence to suggest that, as in the case of larval abortion, follicular degeneration causes an acceleration in the production of the next larva. Table III shows several flies which were found to have degenerated follicles in their ovaries. In all of them the follicles are more advanced than those in normal females of the same age ; it appears that the removal of an egg follicle from the ovulation sequence by degeneration causes the development of the subsequent follicles to be accelerated.

TABLE III.—*The length of the egg follicles in* Glossina morsitans *showing that follicular degeneration causes an acceleration in the development of the other follicles*

	Length of the egg follicles (mm.)							
	Females showing follicular degeneration				Normal females			
Age of fly (in days)	A	B	C	D	A	B	C	D
15	f1—deg	0·75	f1—deg	0·28	f1—ov	0·31	1·298	0·228
	f2—0·15	—	f2—0·12	—	f2—f.s.	—	—	—
18	f1—ov	0·87	f1—deg	0·27	f1—ov	0·41	1·570	0·263
	f2—0·24	—	f2—0·19	—	f2—0·13	—	—	—
18	f1—deg	0·37	f1—deg	0·28	f1—ov	0·41	1·570	0·263
	f2—0·25	—	f2—0·19	—	f2—0·13	—	—	—
19	f1—deg	f1—ov	f1—deg	0·73	f1—ov	0·41	1·600	0·230
	f2—0·31	—	f2—0·25	—	f2—0·15	—	—	—

deg, degenerated follicle.
ov, egg ovulated.
f.s., first signs of descent of follicle from the germarium.
*f*1, first follicle in ovariole.
*f*2, second follicle in ovariole.

VII. DISCUSSION

As many insect-borne parasites undergo a period of development within the arthropod host before they reach an infective form, it is of great practical importance to be able to determine the age of wild-caught insects in order to ascertain the proportion of the population living long enough to transmit pathogens. A reliable method of age determination is also valuable as a check upon the survival of a vector during control programmes.

Early workers (such as Perry (1912) who worked on *Anopheles*) attempted to determine the age of wild-caught insects by the degree of wear and tear of the wings and the loss of scales. This method, however, is not sufficiently accurate because it depends, not only upon the age of the insect, but also upon the conditions under which it has been living. More accurate methods have been worked out by an examination of the female reproductive system. These methods depend upon distinguishing nulliparous from parous females and are reviewed by Gillies (1958) and Bertram and Samarawickrema (1958). The most useful of these methods is that recently developed by Soviet entomologists (Detinova, 1959) for *Anopheles* mosquitoes, which has since been extended to other blood-sucking Diptera and depends upon the recognition of the number of follicular relics (" corpora lutea ") appearing as dilatations of the follicular tubes of the ovarioles. Each dilatation represents the relic of

an egg follicle and is evidence of a gonotrophic cycle, and if the duration of the cycle is known the calendar age of the female may be calculated.

Apart from the mosquitoes, dilatations of the follicular tubes have been found in the ovarioles of several other families of Nematocera and Brachycera. Among the Cyclorrhapha, these structures have been found in parous *Stomoxys calcitrans* L., *Haematobia stimulans* Meigen and *Lyperosia irritans* L. (Kuzina, 1942, 1950), *Hippobosca longipennis* (*capensis*) (Detinova, 1955) and *Musca domestica* L. (Linyova, 1953). As this phenomenon seems so widely spread in the Diptera and evidence of ovulation may be found in the ovarioles of *Glossina morsitans*, it should, theoretically, be possible to determine the number of ovulations or the physiological age of female tsetse flies by an examination of their ovaries.

Existing methods for determining the age composition of *Glossina* populations rely upon the recognition of characters such as the " teneral " state (Jackson, 1940), the duration of which is known to be variable in the field (Jackson, 1946), or the degree of wear and tear, or the colour, of the wings (Jackson 1946 ; Squire, 1950). Whilst the method proposed by Jackson for the age determination of tsetse flies by the degree of wear and tear of the hind margin of the wing appears to be very accurate, it is suggested that a method involving the examination of the ovaries could possibly be more useful as it would also provide information about the number of larvae produced by the flies before capture. It is of interest to note that Mr. W. H. Potts dissected some of the flies recaptured in Jackson's experiment (Jackson, 1946) and examined their ovaries. Two flies, one 18 and the other 20 days old, were found to have a third instar larva with white polypneustic lobes in the uterus, a mature egg in the left ovary and the right ovary " undeveloped ". This is the result one would expect from the present laboratory results.

There was considerable variation in the time of ovulation and the deposition of larvae among the flies studied in this investigation ; a proportion of them failed to become inseminated and others showed follicular degeneration and abortion. It is considered that much of this variation is associated with laboratory culture and probably does not occur in the field.

A method for the determination of the physiological age of female *G. morsitans* is suggested in Table IV. This method depends upon the determination of the number of ovarioles showing evidence of ovulation. A complete dilatation of the follicular tube, as seen in mosquitoes, is not formed, so that serial dilatations are never seen in *Glossina morsitans*, and it is impossible to distinguish ovarioles which ovulated twice or more often from those which have ovulated only once. Because of the sequence of ovulation, however, the number of eggs produced by a female may be determined up to the fourth ovulation (at least 40 days) by the number and position of the " funicle stumps ". After the fourth ovulation it is impossible to determine the number of eggs produced. After the first ovulation (eighth or ninth day at 26–27° C.) eggs are ovulated about every ten days and the uterus contains either an egg or a developing larva. For the purposes of ageing flies, therefore, each of the ten-day periods may be sub-divided by the stage of the offspring *in utero*. Before the first ovulation, when the uterus is empty, flies may be aged by the length of the egg follicle in ovariole *A*.

TABLE IV.—*Suggested table for the determination of the age of female* G. morsitans *at* 26–27° C. *by examination of the ovaries and uterine contents*

1. Uterus empty ; largest egg follicle in right ovary. No evidence of ovulation.	
(*a*) Largest follicle less than 1·0 mm.	1–4 days
(*b*) Largest follicle more than 1·0 mm.	4–7 days
(*c*) Fully developed egg, slips out of follicular tube on dissection . .	7–8 days
2. Evidence of one ovulation in right ovary. Largest follicle in left ovary.	
(*a*) Right ovary contains an " open sac ", uterus contains an egg . . .	8–11 days

(*b*) Ditto, uterus contains small larva (first or second instar) . . . 12–14 days
(*c*) Ovariole *A* shows first signs of descent of second follicle and "funicle stump"; small larva in uterus . . . . . 15–16 days
(*d*) Ovariole *A* shows fully descended second follicle and "funicle stump"; uterus contains second or third instar larva (white lobes) . . . 16–18 days
(*e*) Second follicle in ovariole *A* = 0·15 mm.; uterus contains third instar larva (black lobes); left ovary contains fully-developed egg . . 18–19 days
(*f*) Larva deposited, uterus empty . . . . . 19–20 days (Variation in laboratory = 17–25 days)

3. Evidence of two ovulations, one in the right ovary and one in the left; largest follicle in right ovary.
Second cycle . . . . . 20–30 days (approx.)

4. Evidence of three ovulations, two in right ovary, one in left; largest egg follicle in left ovary.
Third cycle . . . . . 30–40 days (approx.)

5. Evidence of four or more ovulations, two in right and two in left ovaries . 40 days or more (approx.)

VIII. Summary

1. Laboratory studies on the ovaries and the ovulation cycle of *Glossina morsitans* Westwood are reported.

2. Each ovary of *G. morsitans* (also of *G. palpalis* Robineau-Desvoidy, *G. brevipalpis* Newstead and *G. pallidipes* Austen) consists of two polytrophic ovarioles each of which contains a single egg follicle.

3. The egg follicles in the four ovarioles develop in a sequence, so that the first and third eggs are ovulated from the right ovary and the second and fourth from the left ovary. The fifth egg develops in a second follicle in the first ovariole of the right ovary.

4. After ovulation the follicular tube resembles an open dilated sac, most of which breaks away leaving a small piece of tissue at the posterior end of the follicular tube which is evidence of ovulation. The second follicle descends from the germarium several days after the ovulation of the first egg.

5. It is shown that in virgin *G. morsitans* there is little tendency to retain mature eggs within the ovary, but egg follicles in virgin flies are significantly smaller than those in pregnant flies. This may be associated with the larger blood-meals taken by pregnant females.

6. Some egg follicles are shown to degenerate, the degeneration products being passed out of the ovariole, leaving a well-defined dilatation in the follicular tube. Follicular degeneration results in a gap in the production of larvae and probably causes an acceleration in the development of the next egg.

7. It is suggested that a method of age determination for wild-caught female tsetse flies could be based upon an examination of the ovarioles for evidence of ovulation. This, coupled with the stage of the offspring in the uterus, could be used to determine the physiological age of females up to the ovulation of the fourth egg on about the 40th day.

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X. References

Bertram, D. S., 1959, *The ovary and ovariole of mosquitoes.* Course in advanced entomological techniques applied to Malaria eradication. World Health Organization, WHO/Mal./238. London, April 1959.

—— and Samarawickrema, W. A., 1958, Age determination for individual *Mansonioides* mosquitoes. *Nature, Lond.* **182** : 444.

Buxton, P. A., 1955, *The Natural History of Tsetse Flies.* (London School of Hygiene and Tropical Medicine, Memoir No. 10).

—— and Lewis, D. J., 1934, Climate and tsetse flies : laboratory studies on *Glossina submorsitans* and *tachinoides. Philos. Trans.* (B) **224** : 175–240.

Detinova, T. S., 1955, Age changes in the ovaries of *Hippobosca capensis. Dokl. Acad. Sci. USSR* **5** : 937. [Quoted from Detinova, 1959.]

—— 1959, *Age grouping methods in Diptera of medical importance.* Course in advanced entomological techniques applied to Malaria eradication. World Health Organization, WHO/Mal./238. London, April 1959.

Foster, R., 1957, Observations on laboratory colonies of the tsetse flies *Glossina morsitans* Westw. and *Glossina austeni* Newstead. *Parasitology* **47** : 361–74.

Gillies, M. T., 1958, A review of some recent Russian publications on the technique of age determination in *Anopheles. Trop. Dis. Bull.* **55** : 713.

Hagen, H. R., 1951, *Embryology of the Viviparous Insects.* New York.

Jackson, C. H. N., 1936, Some new methods in the study of *Glossina morsitans. Proc. zool. Soc. Lond.* **1936** : 811–96.

—— 1940, The analysis of a tsetse fly population. *Ann. Eugen., Camb.* **10** : 332–69.

—— 1946, An artificially isolated generation of tsetse flies (Diptera). *Bull. ent. Res.* **37** : 291–9.

—— 1949, The biology of tsetse flies. *Biol. Rev.* **24** : 174–99.

Kuzina, O. S., 1942, On the gonotrophic relationships in horse-flies (*Stomoxys calcitrans* and *Haematobia stimulans*). *Med. Parasitol., Moscow* **11** : 70. [Quoted from Detinova, 1959.]

—— 1950, Comparative parasitological and ecological observations on horse-flies (*Stomoxys calcitrans, Haematobia stimulans* and *Lyperosia irritans*). Ectoparasites, Part II : 139. Moscow Society of Naturalists . [Quoted from Detinova, 1959.]

Lewis, D. J., 1958, The recognition of nulliparous and parous *Anopheles gambiae* by examining the ovarioles. *Trans. R. Soc. trop. Med. Hyg.* **52** : 456–61.

Linyova, U. A., 1953, Methods of assessment of the physiological age of females of *Musca domestica. Med. Parasitol., Moscow* **22** : 69. [Quoted from Detinova, 1959.]

Mellanby, H., 1937, Experimental work on reproduction in the tsetse fly, *Glossina palpalis. Parasitology* **29** : 131–41.

Mellanby, K., 1936, Experimental work with the tsetse fly, *Glossina palpalis*, in Uganda. *Bull. ent. Res.* **27** : 611–33.

—— 1937, The reproductive cycle in *Glossina morsitans* and *Glossina swynnertoni. Parasitology* **29** : 142.

Perry, R. L., 1912, Malaria in the Jeypore Hill Tract and adjoining coastland. *Paludism* **5** : 32.

Potts, W. H., 1933, Observations on *Glossina morsitans* Westw. in East Africa. *Bull. ent. Res.* **24** : 293–300.

Pratt, H. S., 1899, The anatomy of the female genital tract of the Pupipara as observed in *Melophagus ovinus. Z. wiss. Zool.* **66** : 16–42.

Roubaud, E., 1909, La *Glossina palpalis* R.–Desv.: sa biologie, son rôle dans l'éteologie des trypanosomiases. *Thesis No.* 1344, University of Paris.

Saunders, D. S., 1960, Ovaries of *Glossina morsitans. Nature, Lond.* **185** : 121–2.

Squire, F. A., 1950, Age grouping of tsetse flies as an aid in the study of their bionomics. *Ibid.* **165** : 307–8.

Stuhlman, F., 1907, Beiträge zur Kenntnis der Tsetsefliege (*Glossina fusca* and *Gl. tachinoides*). *Arb. GesundhAmt., Berl.* **26** : 301–83.

Teesdale, C., 1940, Fertilization in the tsetse fly, *Glossina palpalis*, in a population of low density. *J. Anim. Ecol.* **9** : 24–26.

VANDERPLANK, F. L., 1947, Experiments in the hybridisation of tsetse-flies (*Glossina*, Diptera). *Trans. R. ent. Soc. Lond.* **98** : 1–18.

—— 1948, Experiments in cross-breeding tsetse flies (*Glossina* species). *Ann. trop. Med. Parasit.* **42** : 131–52.

VERHEIN, A., 1921, Die Eibildung der Musciden. *Zool. Jb.* (*Anat.*) **42** : 149–212.

WEITZ, B. and JACKSON, C. H. N., 1955, Host animals of *Glossina morsitans* at Daga-Iloi. *Bull. ent. Res.* **46** : 531–8.